PATENT SPECIFICATION

631,507

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PROVISIONAL SPECIFICATION.

Improvements in or relating to processes for the preparation of Streptomycin

We, THE DISTILLERS COMPANY LIMITED, a British Company, of 12, Torphichen Street. carried out without impairing the titre of the resulting streptomycin solution by

SPECIFICATION NO. 631,507

By a direction given under Section 17(1) of the Patents Act 1949 this application proceeded in the name of The Distillers Company Limited, of 12, Torpichen Street, Edinburgh 3, Scotland, a British company.

THE PATENT OFFICE,
8th April, 1950

DS 31757/1(8)/3366 150 3/50 R

... with streptomyces griseus.

In the large scale fermentations of organic-nitrogenous substances such as peptone-containing media under aerobic conditions, a persistent foam occurs, due apparently, to the increasing degradation of the albuminoid matter in the culture medium. This foam formation makes further aeration and the handling of the broth extremely difficult.

In order to prevent the formation of foam, anti-foam agents have been used. In breweries, for instance, milk has been used with success for said purpose. In other cases, such as for instance in the production of penicillin, by the fermentation of suitable media with penicillium notatum or similar micro-organisms, the addition of small amounts of arachis oil and/or high molecular weight monohydric alcohols has given satisfactory results as regards the prevention of troublesome foam.

When said anti-foam agents were tested in the production of streptomycin, it was found, however, that although preventing the formation of foam, they proved to be useless in practice because their application results in a considerable depression of the streptomycin titre in the fermented broth, caused apparently by reducing the production of streptomycin therein.

It has now been found that the process for the production of streptomycin can be

The amount of the silicone compound necessary for producing the desired effect of substantially suppressing the formation of foam during the fermentation process may vary within wide limits. Very small quantities generally suffice to substantially prevent the formation of foam. It has been found that the addition to the broth to be fermented of an amount of 2 to 3 parts by weight of the silicone compound to one million parts by volume of the broth, gives satisfactory results. On the other hand, a slightly larger amount of the silicone compound appears to have no deleterious effect on the formation or the content of streptomycin in the broth.

It has been found, furthermore, that whilst the hitherto used anti-foaming agents such as arachis oil and/or octadecanol become ineffective in the course of the fermentation and therefore require to be replenished at intervals—a procedure which entails the risk of contaminating the broth by obnoxious micro-organisms—the silicone anti-foaming compounds added at the start to the broth remain effective during the fermentation process. In consequence, when the required amount of the silicone compound is introduced into the broth to be fermented and sterilised therewith before inocculation in the ordinary way, no further addition becomes necessary. The possibility of an

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infection of the culture by the implementation of the anti-foaming agent is thus completely obviated. It is, therefore, an alternative method of carrying out the invention to add the total amount of anti-foam silicone compound required to prevent the formation of foam to the culture medium before the latter is sterilised, subsequently sterilise the broth, inoculate said broth with the suitable micro-organism, and proceed with the fermentation to the end whilst sterile air is passed through said broth without introducing additional amounts of the silicone compound. The fermentation may be carried out while mechanically agitating the culture broth.

The following example illustrates the manner in which the process of the invention may be carried out. The percentages quoted in the example are by weight unless otherwise indicated.

EXAMPLE.

To 2000 cc. of an aqueous culture medium containing:

Peptone	– – –	0.5% by weight
Meat extract	– – –	0.3% „ „
Glucose	– – –	1.0% „ „
Sodium chloride	– –	0.5% „ „
$MgSO_4.7\ H_2O$	– –	0.025% „
$FeSO_4.7\ H_2O$	– –	0.001% „
Water	– – –	rest

is added 0.001% of "DC Anti-foam A" and the medium is then sterilised. The fermentation medium is inoculated with 200 cc. of the same medium on which Streptomyces griseus had been grown after seeding with a spore suspension.

The fermentation is carried out at 28° C. during 5 days whilst the culture medium is stirred vigorously and aerated with sterile air at the rate of 2 litres per minutes. At the end of said 5 days the streptomycin titre of the resulting broth amounted to 153 u/cc.

When a culture medium of a similar composition to which was added before sterilisation 0.25% arachis oil containing 3% of octadecanol in the place of the silicone anti-foam agent, was treated under identical conditions the titre obtained was only 6 u/cc.

The use of the same anti-foam agent, arachis oil, in conjunction with octadecanol, when added in a concentration of 0.1% after the fermentation had been proceeding for some time and after foaming had started in the fermentation vessel resulted in a broth with a titre of 51 u/cc.

The streptomycin may be recovered from the broth in the customary manner, for instance by adsorption with subsequent elution or the like.

Dated this 18th day of September, 1947.

N. F. BAKER,
Agent for the Applicants.

COMPLETE SPECIFICATION.

Improvements in or relating to processes for the preparation of Streptomycin.

We, THE DISTILLERS COMPANY LIMITED, a British Company, of 12, Torphichen Street, Edinburgh, Scotland, and PHILIP DALTON COPPOCK, a British Subject, of the Company's Research and Development Department, Great Burgh, Epsom, Surrey, do hereby declare the nature of this invention and in what manner the same is to be performed, to be particularly described and ascertained in and by the following statement:—

The present invention relates to an improvement in the manufacture of streptomycin, and refers in particular to the process of producing streptomycin by fermenting suitable nutrient media by means of micro-organisms such as Streptomyces griseus.

In the large fermentations of organic nitrogenous substances such as peptone-containing media under aerobic conditions, a persistent foam occurs, due apparently, to the increasing degradation of the albuminoid matter in the culture media. This foam formation makes further aeration and the handling of the broth extremely difficult.

In order to prevent the formation of foam, anti-foam agents have been used. In breweries, for instance, milk has been used with success for said purpose. In other cases, such as for instance in the production of penicillin, by the fermentation of suitable media with penicillium notatum or similar micro-organisms, the addition of small amounts of arachis oil and/or high-molecular weight mono-hydric alcohols has given satisfactory results as regards the prevention of troublesome foam.

When said anti-foam agents were tested in the production of streptomycin, it was found, however, that although preventing the formation of foam, they proved to be useless in practice because their application results in a considerable depression of the streptomycin titre in the fermented broth, caused apparently by reducing the production of streptomycin therein.

It has now been found that the process

for the production of streptomycin can be carried out without impairing the titre of the resulting streptomycin solution by effecting the fermentation in the presence of a silicone anti-foaming agent.

Accordingly the process of the present invention for the production of streptomycin solutions comprises fermenting a culture medium suitable for streptomycin production by means of streptomycin-producing micro-organisms such as Streptomyces griseus under aerobic conditions in the presence of a silicone anti-foaming compound, as herein defined.

By the term silicone anti-foaming compound as used in the Specification and claims is meant the polymeric dihydrocarbon siloxanes, such as the liquid polymeric dimethyl siloxanes and the material sold under the Trade Name "D.C. Antifoam A."

Dimethyl siloxanes which are especially suitable for the process of the present invention are those of the following general formulae:

I $(CH_3)_2Si\text{-}O\text{-}[(CH_3)_2Si\text{-}O\text{-}]_x\text{-}(CH_3)_2Si\text{-}O$ (the first Si and the final O being joined)

where x is zero or an integer; or

II $R\text{-}(CH_3)_2Si\text{-}O\text{-}[(CH_3)_2Si\text{-}O\text{-}]_y\text{-}(CH_3)_2Si\text{-}R$

where y is an integer greater than 20, and R represents an alkyl, preferably methyl, or a hydroxyl group.

Examples of compounds of the above formulae which may be used include decamethyl cyclopentasiloxane, octamethyl cyclotetrasiloxane and the polymeric dimethyl siloxane diols of the formula II above where R is a hydroxyl group, which can be prepared by the hydrolysis of dimethyl dichloro silane. It should be noted that the present invention includes the use of a mixture of these polymeric dimethyl siloxane compounds as well as the use of the pure materials themselves.

The amount of the silicone compound necessary for producing the desired effect of substantially suppressing the formation of foam during the fermentation process may vary within wide limits although very small quantities have been found adequate to prevent the formation of foam substantially completely. It has been found that the addition to the broth to be fermented of an amount of about 2 to 40 parts by weight of the said silicone to one million parts by volume of the broth is satisfactory, said parts by weight and parts by volume bearing the same relation to each other as do grams to millilitres. On the other hand, a slightly larger amount of the silicone compound appears to have no inhibiting effect on the production of streptomycin, but of course, it is obviously uneconomic to employ a larger amount of the silicone compound than is necessary, and in general the amount of silicone compound used need not be in excess of 40 parts by weight per million parts by volume of broth.

It has been found, futhermore, that whilst the hitherto used anti-foaming agents, such as arachis oil, octadecanol, lard oil and maize oil, become ineffective in the course of the fermentation and therefore require to be replenished at intervals—a procedure which entails the risk of contaminating the broth by obnoxious micro-organisms—the said silicone anti-foaming compounds added at the start to the broth remain effective during the whole fermentation process. In consequence, when the required amount of the silicone compound is introduced into the broth to be fermented and sterilised therewith before innoculation in the ordinary way, no further addition becomes necessary. The possibility of an infection of the culture by the implementation of the anti-foaming agent is thus completely obviated. It is, therefore, an alternative method of carrying out the invention to add the total amount of anti-foam silicone compound required to prevent the formation of foam to the culture medium before the latter is sterilised, subsequently sterilise the broth, inoculate said broth with the suitable micro-organism, and proceed with the fermentation to the end whilst sterile air is passed through said broth without introducing additional amounts of the silicone compound. The fermentation may be carried out while mechanically agitating the culture broth.

The following examples illustrate the ways in which the process of the invention may be carried out. The percentages quoted in the examples are by weight unless otherwise indicated, and the quoted parts by weight and parts by volume bear the same relation to each other as do grams to millilitres.

EXAMPLE 1.

To 2000 cc. of an aqueous culture medium containing:

Peptone	– – – – 0.5%	by weight
Meat extract	– – – 0.3%	,, ,,
Glucose	– – – 1.0%	,, ,,
Sodium chloride	– – 0.5%	,, ,,
$MgSO_4.7H_2O$	– – 0.025%	,,
$FeSO_4.7H_2O$	– – 0.001%	,,
Water	– – – – rest	

is added 0.001% of "DC Anti-foam A" and the medium is then sterilised. The fermentation medium is inoculated with 200 cc. of the same medium on which Streptomyces griseus had been grown after seeding with a spore suspension.

The fermentation is carried out at 28° C. during 5 days whilst the culture medium is stirred vigorously and aerated with sterile air at the rate of 2 litres per minute. At the end of said 5 days the streptomycin titre of the resulting broth amounted to 153 u/cc.

For purposes of comparison when a culture

medium of a similar composition, to which was added before sterilisation 0.25% arachis oil containing 3% of the octadecanol in the place of the "DC Anti-foam A," was treated under identical conditions the titre obtained was only 6u/cc. Similarly the use of the same anti-foam agent, arachis oil, in conjunction with octadecanol, when added in a concentration of 0.1% after the fermentation had been proceeding for some time and after foaming had started in the fermentation vessel resulted in a broth with a titre of only 51 u/cc.

The streptomycin may be recovered from the broth in the customary manner, for instance by adsorption with subsequent elution or the like.

Example 2.

For comparative purposes a series of experiments were carried out employing maize oil, lard oil and "D.C. Antifoam A" as anti-foam agents. The fermentations were carried out as described in Example 1, employing various concentrations of the anti-foams.

The results obtained were as follows:

Antifoam	*Amount as % by weight of the medium.*	*Average titre per ml. of broth*
Maize oil	0.3	31.25
" "	0.5	33.4
" "	0.7	8.3
Lard Oil	0.1	77
" "	0.3	77
" "	0.5	54
D.C. Anti-foam	30 part by weight per million parts by volume of the medium.	176

It was found that the maize oil did not completely control foaming in any of the concentrations employed; the lard oil was only effective to control foaming in concentrations in excess of 1%. The "D.C. Anti-foam A" in the amount employed completely controlled foaming.

In similar experiments employing 0.5% by weight of milk as the anti-foam agent, it was found that although foaming was controlled, the streptomycin production in those experiments where milk was used was less than half that obtained in the control experiments where no anti-foam was employed.

Example 3.

A set of six parallel fermentations were carried out, in each of which experiments 2 litres of an aqueous culture medium containing:—

Bacto peptone	0.5%
Yeast extract	0.3%
Glucose	1.0%
Sodium chloride	0.5%
Magnesium sulphate	0.025%
Ferrous sulphate	0.001%

was inoculated with an aqueous suspension of spores of Streptomyces griseus, and the fermentation carried out at 29° C. with aeration at the rate of 1½ volumes of air per volume of medium per minute. For comparative purposes in one experiment no anti-foam was incorporated in the medium whereas in the other five experiments the silicone anti-foam compound shown in column 1 of the table below was incorporated in the medium in an amount comprising 20 parts by weight of the anti-foam per million parts by volume of the medium in accordance with the process of the present invention. The streptomycin titre of the broth obtained is given in column 2 below:

Exp.	*Column 1.*	*Column 2.*
1	Polymeric dimethyl siloxane doils of B.P. above 250° C. at 2 mm/Hg.	63
2	Polymeric dimethyl siloxane diols of B.P. below 250° C. at 2 mm/Hg.	79
3	Decamthyl cyclopentasiloxane.	70
4	Octamethyl cyclotetrasiloxane	65
5	D.C. Antifoam A	81
6	No anti-foam	62

In experiments 1—5 foaming was completely suppressed. In experiment 6 where no anti-foam was employed substantial foaming occurred.

Example 4.

Five fermentations were carried out wherein 3 litres of an aqueous culture medium containing:—

Fish meal	0.7%
Dried autolysed yeast	0.3%
Glucose	1.0%
Sodium chloride	0.5%
Magnesium sulphate	0.025%
Ferrous sulphate	0.001%

and one of the silicone anti-foams indicated below in an amount comprising 40 parts by weight per million parts by volume of the medium were inoculated with an aqueous

suspension of spores of Streptomyces griseus and the fermentation carried out at 28° C, the medium being stirred at the rate of 900 r.p.m., and air being pumped through the medium at the rate of $1\frac{1}{3}$ volumes of air per volume of medium per minute.

The fermentations were incubated for 4 days and the titre of the resulting broth as units of streptomycin per ml. of broth when using each silicone anti-foam are given in the table below.

Exp.	*Silicone Anti-foaming Compound.*	*Titre.*
1	D.C. Antifoam A.	161
2	Polymeric dimethyl siloxane diols of B.P. above 250° C. at 2 mm /Hg.	138
3	Polymeric dimethyl siloxane diols of B.P. below 250° C. at 2 mm /Hg.	161
4	Decamethyl cyclopentasiloxane.	151
5	Octamethyl cyclotetrasiloxane.	171

In each experiment foaming during the fermentation was completely suppressed.

The polymeric dimethyl siloxane diols employed in Example 3 and 4 are of the formula :

$OH\text{-}(CH_3)_2Si\text{-}O\text{-}[(CH_3)_2Si\text{-}O\text{-}]_x\text{-}(CH_3)_2Si\text{-}OH$

where x is an integer in excess of about 200, and these compounds may be prepared by fractionating the hydrolysis product of dimethyl dichlorosilane.

The liquid silicone anti-foaming compounds which are used according to the process of the present invention may be added to the culture medium either with or without dilution. However, in view of the minute quantity of the silicone compound which is required for each fermentation, it may be advisable to employ a solution or dispersion of the silicone compound in an inert diluent such as petroleum ether.

Having now particularly described and ascertained the nature of our said invention and in what manner the same is to be performed, we declare that what we claim is :—

1. A process for the production of streptomycin by the fermentation of a culture medium suitable for streptomycin production by means of a streptomycin-producing organism such as Streptomyces griseus under aerobic conditions characterised in that the fermentation is carried out in the presence of a silicone anti-foaming compound as hereindefined.

2. A process for the production of streptomycin as claimed in Claim 1, where in the silicone anti-foaming compound is present in an amount comprising 2 to 40 parts by weight per million parts by volume of the culture medium, said parts by weight and parts by volume bearing the same relation to each other as do grams to millilitres.

3. A process for the production of streptomycin as claimed in either of the preceding claims, wherein the silicone anti-foaming compound in admixture with an inert diluent.

4. A process for the production of streptomycin as claimed in any of the preceding claims, wherein the silicone anti-foaming compound employed is a diemthyl siloxane of the following formula :—

$$(CH_3)_2Si\text{-}O\text{-}[(CH_3)_2Si\text{-}O\text{-}]_x\text{-}(CH_3)_2Si\text{-}O$$

where x is zero or an integer.

5. A process for the production of streptomycin as claimed in any of Claims 1—3, wherein the silicone anti-foaming compound employed is a dimethyl siloxane of the following formula

$R\text{-}(CH_3)_2Si\text{-}O\text{-}[(CH_3)_2Si\text{-}O\text{-}]_y\text{-}(CH_3)_2Si\text{-}R$

where y is an integer greater than 20, and R represents an alkyl, preferably methyl, or a hydroxyl group.

6. A process for the production of streptomycin as claimed in any of Claims 1—3, wherein the silicone anti-foaming compound employed is the material sold under the Trade Name "D.C. Antifoam A."

7. A process for the production of streptomycin as described in any of the foregoing Examples 1, 3 and 4.

8. Streptomycin when prepared by the process of any of the preceding claims.

Dated this 13th day of September, 1948.

N. F. BAKER.
Agent for the Applicants.

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